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(54) Title: TOLL-LIKE RECEPTOR 11

(57) Abstract: The invention describes isolated Toll-like receptor 11 ("TLR11") polypeptides as well as isolated variants and fragments thereof and the isolated nucleic acids encoding them. The invention also describes vectors and host cells containing nucleic acid encoding a TLR11 polypeptide and methods for producing a TLR11 polypeptide. Also described are methods for screening for compounds which modulate TLR11 activity and methods of use of TLR11 polypeptides as adjuvants.

TOLL-LIKE RECEPTOR 11

RELATED APPLICATION

This application claims the benefit of the filing date of U.S. Provisional Application number 60/363,621, entitled "TLR 11 Is a Novel Toll-like Receptor", by Sankar Ghosh (filed March 11, 2002). The entire teachings of the referenced Provisional Application are incorporated herein by reference.

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BACKGROUND OF THE INVENTION

Toll-like receptors (TLRs) represent a growing family of transmembrane proteins characterized by multiple copies of leucine-rich repeats in the extracellular domain and a cytoplasmic Toll/interleukin-1 receptor (TIR) motif. TIR motifs of TLRs exhibit significant homology to the intracellular signaling domain of the type I interleukin-1 (IL-1) receptor. TLRs are evolutionarily conserved, and their congeners have been found in insects, plants, and mammals. Drosophila Toll (dToll) was the first member of the TLR family to be identified and was initially characterized as a developmental protein governing the formation of the dorsal-ventral axis in Drosophila (Belvin, MP and Anderson, KV (1996) Annu. Rev. Cell Dev. Biol. 12:393-416). However, subsequent studies revealed that dToll also plays a key role in triggering innate immune responses against fungal infection in adult flies (Anderson, KV (2000) Curr. Opin. Immunol. 12:13-19; Belvin, MP and Anderson, KV (1996) Annu. Rev. Cell Dev. Biol. 12:393-416).

To date, more than ten distinct Toll-like sequences, homologous to the highly conserved cytoplasmic domain sequence of dToll, have been identified in the

largely completed Drosophila genomic sequence. In humans, nine full-length TLR sequences have also been deposited in GenBank, while six other members remain partially characterized (Anderson, KV (2000) Curr. Opin. Immunol. 12:13-19; O'Neill, LA and Greene, C (1998) J. Leukot. Biol. 63:650-7).

The Toll-like receptors (TLRs) are also thought to participate in mechanisms of innate immunity and inflammation acting as pattern recognition receptors (PRRs) for bacteria and other micro-organisms. As PRRs, TLRs recognize invariant molecular structures called pathogen-associated molecular patterns (PAMPs) that are shared by many pathogens but are not expressed by hosts. TLRs are distinguished from other PRRs by their ability to recognize and discriminate between different classes of pathogens (Janeway, CA and Medzhitov, R (1999) Curr. Biol. 9:R879-R882; Anderson, KV (2000) Curr. Opin. Immunol. 12:13-19). Engagement of TLRs by pathogens leads to the activation of innate immune responses, and a major signaling target of the TLRs is activation of the transcription factor NF-κB, a key regulator of immune and inflammatory responses (Ghosh, S, et al (1998) Annu. Rev. Immunol. 16:225-260; May, MJ and Ghosh, S (1998) Immunol. Today 19:80-88; and Karin, M and Ben-Neriah, Y (2000) Annu. Rev. Immunol. 18:621-663). TLR-mediated NF-κB activation is an evolutionarily conserved event that occurs in phylogenetically distinct species ranging from insects to mammals (Anderson, KV (2000) Curr. Opin. Immunol. 12:13-19; O'Neill, LA and Greene, C (1998) J. Leukot. Biol. 63:650-657). TLRs can elicit pro-inflammatory cytokine production and induce expression of cell surface co-stimulatory receptors required for activation of T-cells. Some TLRs may help to co-ordinate interactions between cells of the innate and acquired immune systems to orchestrate an integrated immune response to infection.

SUMMARY OF THE INVENTION

The present invention relates to the discovery of a Toll-like receptor of mammalian origin, termed Toll-like receptor 11 ("TLR11"). TLR11 is a screening target for the identification and development of novel pharmaceutical agents which modulate the activity of the receptor, for example, have immunomodulatory activity.

The invention relates to isolated TLR11 polypeptides. Polypeptide fragments or variants of a TLR11 polypeptide are additional embodiments of this invention. The invention additionally relates to isolated nucleic acids (e.g., DNA, RNA) encoding a TLR11 polypeptide, TLR11 fragments and TLR11 variants. The invention further relates to nucleic acids that are complementary to nucleic acid encoding a TLR11 polypeptide. In certain embodiments, the invention relates to nucleic acid which hybridizes under high stringency conditions to all or a portion of nucleic acid encoding a TLR11 polypeptide.

In certain aspects, the invention provides expression vectors comprising nucleic acid encoding a TLR11 polypeptide. Host cells comprising exogenous nucleic acid (e.g., DNA, RNA) encoding a TLR11 polypeptide, such as host cells containing an expression vector comprising nucleic acid encoding a TLR11 polypeptide, are also the subject of this invention. In another embodiment, the invention relates to a method for producing a TLR11 polypeptide, such as a method of producing a TLR11 polypeptide in isolated host cells containing a vector expressing a TLR11 polypeptide. In certain aspects, the invention relates to an antibody that is specific for a TLR11 polypeptide of the invention.

In certain embodiments, the invention provides a method of screening for compounds which modulate the activity of TLR11. Compounds (e.g., agonists or antagonists) which modulate TLR11 activity are also the subject of this invention. In another aspect, the invention provides a method of treatment for diseases affected by TLR11 activity (e.g., immune or inflammatory disorders) which includes administration of a compound which modulates TLR11 activity.

In another embodiment, the invention relates to a TLR11 polypeptide, nucleic acid encoding a TLR11 polypeptide, or an antibody specific for a TLR11 polypeptide for use as an adjuvant or for use in the manufacture of an adjuvant or vaccine. The invention also relates to compounds which modulate TLR11 activity for use in the manufacture of a medicament for the treatment of diseases affected by TLR11 activity (e.g., immune or inflammatory disorders).

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-E.

Figure 1A shows the cDNA sequence encoding mouse TLR11 (SEQ ID NO: 1).

Figure 1B shows the amino acid sequence of mouse TLR11 (SEQ ID NO: 2). The predicted signal peptide (residues 1 to 30) and the trans-membrane segment (residues 705 to 729) are underlined. Amino acids are represented by their single letter codes.

Figure 1C is an alignment of the amino acid sequence of cytoplasmic domains of known Toll-like receptor family members, mouse TLRs ("mTLR") and a human TLR ("hTLR"), with TLR-11. Alignments were performed using the Clustal algorithm and boxshade. Three regions (box 1, 2 and 3) are conserved across all TIR domains and appear to be important for signaling. The sequences aligned are the amino acids sequences of mTLRs 1-9 (SEQ ID NOS: 3-11), hTLR10 (SEQ ID NO: 12) and mTLR11 (SEQ ID NO: 13).

Figure 1D is a blot depicting multiple tissue Northern analysis to determine the expression pattern of TLR 11 mRNA. TLR11 is predominantly expressed in kidney and liver with significantly lower levels of expression in spleen and heart. A β-actin probe was used as a control for RNA loading.

Figure 1E is a picture depicting the localization of TLR11 mRNA in tissues by in situ hybridization. TLR11 localization is shown by incubation with the antisense riboprobe of TLR11 in medulla (a) and cortex (c) of kidney, and liver (e). Control incubations using TLR11 sense riboprobe were negative (b, d, f).

Figures 2A-F.

Figure 2A is a graph depicting constitutively active TLR11 that activates NF-κB.

Figure 2B is a graph depicting constitutively active TLR11 that activates AP1. 293 cells were transiently transfected with expression vectors for CD4/TLR11 or

CD4/TLR4 fusion constructs. In these constructs the cytosolic domain of the TLRs (the TIR domain) was fused to the extracellular portion of CD4. The amount of DNA transfected was equalized with empty expression vector, which was also used in the control together with either an NF-κB or AP1 luciferase reporter construct. NF-κB and AP1 induced luciferase activity were measured using a luminometer.

Figure 2C is a graph depicting transfection of RAW 264.7 macrophages with a CD4/TLR11 expression vector. The production of TNF-α was detected by immunostaining for cell surface TNF followed by flow cytometry. The dark gray region indicates TNF-α expression in untransfected cells, whereas the light gray line represents TNF-α produced in cells transfected with CD4/TLR11.

Figure 2D is a graph depicting dominant-negative MyD88 (DN-MyD88) construct that inhibits CD4/TLR11 mediated NF-κB activation.

Figure 2E is a graph depicting dominant-negative IRAK (DN-IRAK) and dominant-negative TRAF6 (DN-TRAF6) constructs that inhibit CD4/TLR11 mediated NF-κB activation. 293-luc cells, stably transfected cells with the NF-κB luciferase reporter construct, was co-transfected with CD4/TLR11 and wild-type or dominant-negative versions of MyD88, IRAK and TRAF6.

Figure 2F is a graph depicting tollip that inhibits CD4/TLR11 induced NF-κB activation. Tollip, a physiological inhibitor of Toll-signaling, was cotransfected into 293-luc cells along with CD4/TLR11.

Figures 3A-B.

Figure 3A is a picture depicting an immunoblot confirming expression of TLR11. Six different, independently derived cell lines stably expressing TLR11/pFlag in 293 cells transfected with the κB-luciferase reporter (293-luc cells) were obtained.

Figure 3B are graphs depicting cell surface expression of TLR11 in the stable cell line. Cell surface expression of TLR11 in the stable cell line was detected using FACS. The dark gray region indicates untransfected cells, whereas the light gray line indicates cells transfected with TLR11/pFlag.

Figures 4A-B.

Figure 4A is a graph depicting luciferase activity in cells following treatment with the indicated agents. 293-luc cells were transiently transfected with TLR2, TLR4, TLR5, TLR11 or empty expression vectors. Luciferase activity in cells was measured following treatment with 100 ng ml-1 PGN, 100 ng ml-1 LPS, 100 ng ml-1 Flagellin, 100 ng ml-1 dsRNA, 100 ng ml-1 CpG DNA, or untreated (control) cells.

Figure 4B is a graph depicting luciferase activity in cells following treatment with the indicated saturated bacterial cultures or LB alone. The 293-luc cells stably transfected with TLR2 or TLR11 were treated with 70μl ml-1 of heat-killed supernatant from the indicated saturated bacterial cultures or LB alone (control). Data are representative of three independent experiments.

Figure 5 is a schematic of the putative conserved domains of TLR. The schematic is a comparison of mouse TLRs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides nucleic acids and the polypeptides encoded thereby relating to a Toll-like receptor, termed Toll-like receptor 11 ("TLR11"). Described herein are isolated TLR11 polypeptides, fragments and variants thereof; isolated nucleic acids (e.g., DNA, RNA) encoding TLR11 polypeptides, fragments and variants thereof; methods of producing TLR11 polypeptides; and methods in which TLR11 peptides are used. Such nucleic acids and polypeptides are of eukaryotic origin, such as mammalian origin (e.g. mouse, human).

In some aspects, the invention provides TLR11 nucleic acid sequences and proteins encoded thereby, as well as oligonucleotides derived from the nucleic acid sequences, antibodies that bind the encoded proteins, screening assays to identify agents that modulate TLR11 activity and/or biological events affected by TLR11, and compounds that modulate TLR11 activity and/or biological events affected by TLR11. These compounds may be used in the treatment and/or prophylaxis of inflammatory diseases; cardiovascular diseases; systemic infections; autoimmune diseases, such as asthma; rhinitis; chronic obstructive pulmonary disease (COPD); emphysema; inflammatory bowel diseases such as ulcerative colitis and Crohn's disease; rheumatoid arthritis; osteoarthritis; psoriasis; Alzheimers disease; atherosclerosis; viral, fungal and bacterial infections, including urinary tract infections; septic shock syndrome associated with systemic infection involving gram positive and gram negative bacteria; diabetes; and Multiple Sclerosis. These agents may also be used as immunoadjuvants to enhance or alter the immune response in vaccine therapy.

In one aspect, the invention provides an isolated nucleic acid comprising a nucleic acid which hybridizes under high stringency conditions to a nucleic acid having the sequence of SEQ ID NO: 1 or a sequence complementary thereto. In a further embodiment, the invention is an isolated nucleic acid that is at least about 70%, 80%, 90%, 95%, 97-98%, or greater than 99% identical to a sequence corresponding to at least about 12, at least about 15, at least about 25, at least about 40, at least about 100, at least about 300, at least about 500, at least about 1000, or at least about 2500 consecutive nucleotides up to the full length of SEQ ID NO: 1, or a sequence complementary thereto. In specific embodiments, nucleic acids exhibit one of the foregoing levels of identity to SEQ ID NO: 1 and encode polypeptides

that also exhibit substantially the same activity or function as TLR11 encoded by SEQ ID NO: 1.

Isolated nucleic acids of the present invention are relatively free from unrelated nucleic acids as well as contaminating polypeptides, nucleic acids and other cellular material that normally are associated with the nucleic acid in a cell or that are associated with the nucleic acid in a library.

In other embodiments, the invention provides expression vectors (constructs) comprising: (a) a nucleic acid which hybridizes under high stringency conditions to a sequence of SEQ ID NO: 1, or a nucleotide sequence that is at least about 70%, 80%, 90%, 95%, 97-98%, or greater than 99% identical to a sequence that is at least about 12, at least about 15, at least about 25, at least about 40, at least about 100, at least about 300, at least about 500, at least about 1000, or at least about 2500 consecutive nucleotides up to the full length of SEQ ID NO: 1, or a sequence complementary thereto, and (b) a transcriptional regulatory sequence operably linked to the nucleotide sequence. In certain embodiments, an expression vector of the present invention additionally comprises a transcriptional regulatory sequence, e.g., at least one of a transcriptional promoter or transcriptional enhancer sequence, which regulatory sequence is operably linked to the TLR11 sequence. In another embodiment, the nucleic acid may be included in an expression vector capable of replicating in and expressing the encoded TLR11 polypeptide in a prokaryotic or eukaryotic cell. In a related embodiment, the invention provides a host cell transfected with the expression vector.

Any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a TLR11 polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating factors, the polyhedron

promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

As will be apparent, the subject gene constructs can be used to cause expression of the subject TLR11 polypeptides in cells propagated in culture, e.g., to produce proteins or polypeptides, including fusion proteins or polypeptides, for purification.

This invention also pertains to a host cell transfected with a recombinant gene comprising a coding sequence for one or more of the subject TLR11 polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the present invention may be expressed in bacterial cells, such as E. coli, insect cells (e.g., using a baculovirus expression system), yeast, avian, or mammalian cells (e.g., human cells such as HEK293, HeLa).

Accordingly, the present invention further pertains to methods of producing the subject TLR11 polypeptides. For example, a host cell transfected with an expression vector encoding a TLR11 polypeptide can be cultured under appropriate conditions to allow expression of the polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the polypeptide may be retained cytoplasmically and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The polypeptide can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for particular epitopes of the polypeptide. In a preferred embodiment, the TLR11 polypeptide is a fusion protein containing a domain which facilitates its purification, such as a TLR11-GST fusion

protein, TLR11-intein fusion protein, TLR11-cellulose binding domain fusion protein, and TLR11-polyhistidine fusion protein.

A nucleotide sequence encoding a TLR11 polypeptide can be used to produce a recombinant form of the protein via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial) cells, are standard procedures.

A recombinant TLR11 nucleic acid can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells, or both. Expression vehicles for production of recombinant TLR11 polypeptides include plasmids and other vectors. For instance, suitable vectors for the expression of a TLR11 polypeptide include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as E. coli.

A number of vectors exist for the expression of recombinant proteins in yeast. For instance, YEP24, YIP5, YEP51, YEP52, pYES2, and YRP17 are cloning and expression vehicles useful in the introduction of genetic constructs into S. cerevisiae. These vectors can replicate in E. coli due to the presence of the pBR322 ori, and in S. cerevisiae due to the replication determinant of the yeast 2 micron plasmid. In addition, drug resistance markers such as ampicillin can be used.

Certain mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient

expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. In some instances, it may be desirable to express the recombinant TLR11 polypeptide by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

Alternatively, the coding sequences for the polypeptide can be incorporated as a part of a fusion gene including a nucleotide sequence encoding a different polypeptide. This type of expression system can be useful under conditions where it is desirable, e.g., to produce an immunogenic fragment of a TLR11 polypeptide. For example, the VP6 capsid protein of rotavirus can be used as an immunologic carrier protein for portions of polypeptide, either in the monomeric form or in the form of a viral particle. The nucleic acid sequences corresponding to the portion of the TLR11 polypeptide to which antibodies are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia virus structural protein to produce a set of recombinant viruses expressing fusion proteins comprising a portion of the protein as part of the virion. The Hepatitis B surface antigen can also be utilized in this role as well. Similarly, chimeric constructs coding for fusion proteins containing a portion of a TLR11 polypeptide and the poliovirus capsid protein can be created to enhance immunogenicity.

In yet another embodiment, the invention provides a substantially pure nucleic acid which hybridizes under high stringency conditions to a nucleic acid probe that comprises at least about 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides up to the full length of SEQ ID NO:1, or a sequence complementary thereto or up to the full length of the gene of which said sequence is a fragment. The invention also provides an antisense oligonucleotide analog which hybridizes under stringent conditions to at least 12, at least 25, or at least 50 consecutive nucleotides up to the full length of SEQ ID NO:1, or a sequence complementary thereto.

One of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For

example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, the invention provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature. In another embodiment, the invention provides nucleic acids which hybridize under high stringency conditions of 0.5 x SSC at 60°C followed by 2 washes at 0.5 x SSC at 60°C.

In a further embodiment, the invention provides a nucleic acid comprising a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2, or a nucleic acid complementary thereto. In a further embodiment, the encoded amino acid sequence is at least about 70%, 80%, 90%, 95%, or 97-98%, or greater than 99% identical to a sequence corresponding to at least about 12, at least about 15, at least about 25, or at least about 40, at least about 100, at least about 200, at least about 300, at least about 400 or at least about 500 consecutive amino acid residues up to the full length of SEQ ID NO: 2.

Nucleic acids of the invention further include nucleic acids that comprise variants of SEQ ID NO:1. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; and will, therefore, include coding sequences that differ from the nucleotide sequence of the coding sequence designated in SEQ ID NO:1, e.g., due to the degeneracy of the genetic code. In other embodiments, variants will also include sequences that will hybridize under highly stringent conditions to a nucleotide sequence of a coding sequence designated in SEQ ID NO: 1.

Isolated nucleic acids which differ from SEQ ID NO:1 due to degeneracy in the genetic code are also within the scope of the invention. For example, a number of amino acids are designated by more than one triplet. Codons that specify the

same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. All such nucleotide variations and resulting amino acid polymorphisms are within the scope of this invention.

In another embodiment, the invention provides a probe or primer (e.g., DNA, RNA) which hybridizes under stringent conditions to at least about 12, at least about 15, at least about 25, or at least about 40 consecutive nucleotides of sense or antisense sequence selected from SEQ ID NO: 1, or a sequence complementary thereto. In certain embodiments, a probe of the present invention hybridizes to a characteristic region of SEQ. ID. NO: 1 and is useful to identify additional toll-like receptors. The probe may include a detachable label, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. The invention further provides arrays of at least about 10, at least about 25, at least about 50, or at least about 100 different probes as described above attached to a solid support. Such arrays are useful to assess samples (e.g., tissues, blood, cells) for the presence of TLR11 nucleic acids (e.g., TLR11 mRNA).

Optionally, a TLR11 nucleic acid of the invention will genetically complement a partial or complete TLR11 loss of function phenotype in a cell. For example, a TLR11 nucleic acid of the invention may be expressed in a cell in which endogenous TLR11 has been reduced by RNAi, and the introduced TLR11 nucleic acid will mitigate a phenotype resulting from the RNAi. The term "RNA interference" or "RNAi" refers to any method by which expression of a gene or gene product is decreased by introducing into a target cell one or more double-stranded RNAs which are homologous to the gene of interest (particularly to the messenger RNA of the gene of interest).

Another aspect of the invention relates to TLR11 nucleic acids that are used for antisense, RNAi or ribozymes. As used herein, nucleic acid therapy refers to administration or in situ generation of a nucleic acid or a derivative thereof which specifically hybridizes (e.g., binds) under cellular conditions with the cellular mRNA and/or genomic DNA encoding one of the subject TLR11 polypeptides so as to inhibit production of that protein, e.g., by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix.

A nucleic acid therapy construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes a TLR11 polypeptide. Alternatively, the construct is an oligonucleotide which is generated ex vivo and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences encoding a TLR11 polypeptide. Such oligonucleotide probes are optionally modified oligonucleotides which are resistant to endogenous nucleases, e.g., exonucleases and/or endonucleases, and is therefore stable in vivo. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in nucleic acid therapy have been reviewed, for example, by van der Krol et al., (1988) Biotechniques 6:958-976; and Stein et al., (1988) Cancer Res 48:2659-2668. Nucleic acid constructs of the invention are useful in therapeutic, diagnostic, and research contexts.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the TLR11 DNA or RNA sequences to which they specifically bind, such as for determining the level of expression of a gene of the invention or for determining whether a gene of the invention contains a genetic lesion.

In another aspect, the invention provides polypeptides. In one embodiment, the invention pertains to a polypeptide encoded by a nucleic acid which hybridizes

under stringent conditions to a nucleic acid nucleic acid of SEQ ID NO: 1, a sequence complementary thereto, or a fragment encoding an amino acid sequence comprising at least about 25, or at least about 40 amino acid residues thereof.

In another embodiment, the TLR11 polypeptide comprises a sequence that is identical with or homologous to SEQ ID NO: 2. For instance, a TLR11 polypeptide preferably has an amino acid sequence at least 70% identical to a polypeptide represented by SEQ ID NO: 2 or an amino acid sequence that is 80%, 90% or 95% identical thereto. The TLR11 polypeptide can be full length, such as the polypeptide represented by the amino acid sequence in SEQ ID NO: 2 or it can comprise a fragment of, for instance, at least 5, 10, 20, 50, 100, 150, 200, 250, 300, 400 or 500 or more amino acid residues in length.

In another embodiment, the invention features a purified or recombinant polypeptide fragment of a TLR11 polypeptide, which polypeptide has the ability to modulate, e.g., mimic or antagonize, an activity of a wild-type TLR11 protein. Preferably, the polypeptide fragment comprises a sequence identical or homologous to the amino acid sequence designated in SEQ ID NO: 2.

Moreover, as described below, the TLR11 polypeptide can be either an agonist or alternatively, an antagonist of a biological activity of a naturally occurring form of the protein, e.g., the polypeptide is able to modulate the intrinsic biological activity of a TLR11 protein or a TLR11 complex, such as activation of NF-κB or the production of cytokines (e.g., TNF-α).

The present invention also relates to chimeric molecules, such as fusion proteins, that comprise all or a portion of a TLR11 polypeptide and a second polypeptide that is a heterologous (not a TLR11 polypeptide), such as the extracellular domain of CD4 or an epitope tag, such as a Flag or myc epitope tag).

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature.

Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-

ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992).

The present invention also makes available isolated and/or purified forms of the subject TLR11 polypeptides, which are isolated from, or otherwise substantially free of, other intracellular proteins which might normally be associated with the protein or a particular complex including the protein. TLR11 polypeptides which are recombinantly produced (e.g., by recombinant DNA methods) or chemically synthesized are also the subject of this invention.

Optionally, a TLR11 polypeptide of the invention will function in place of an endogenous TLR11 polypeptide, for example by mitigating a partial or complete TLR11 loss of function phenotype in a cell. For example, a TLR11 polypeptide of the invention may be produced in a cell in which endogenous TLR11 has been reduced by RNAi, and the introduced TLR11 polypeptide will mitigate a phenotype resulting from the RNAi.

Variants and fragments of a TLR11 polypeptide may have enhanced activity or constitutive activity, or, alternatively, act to prevent TLR11 polypeptides from performing one or more functions. For example, a truncated form lacking one or more domain may have a dominant negative effect.

Another aspect of the invention relates to polypeptides derived from a full-length TLR11 polypeptide. Isolated peptidyl portions of the subject proteins can be obtained by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding such polypeptides. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, the subject protein can be arbitrarily divided into fragments of desired length with no overlap of the

fragments, or preferably divided into overlapping fragments of a desired length. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments which can function as either agonists or antagonists of the formation of a specific protein complex, or more generally of a TLR11 complex, such as by microinjection assays.

It is also possible to modify the structure of the subject TLR11 polypeptides for such purposes as enhancing therapeutic or prophylactic efficacy, or stability (e.g., ex vivo shelf life and resistance to proteolytic degradation in vivo). Such modified polypeptides, when designed to retain at least one activity of the naturally-occurring form of the protein, are considered functional equivalents of the TLR11 polypeptides described in more detail herein. Such modified polypeptides include peptide mimetics. Peptide mimetics include chemically modified peptides and peptide-like molecules containing non-naturally occurring amino acids. Modified polypeptides can also be produced, for instance, by amino acid substitution, deletion, or addition.

For instance, it is reasonable to expect, for example, that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (i.e. conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide results in a functional homolog can be readily determined by assessing the ability of the variant polypeptide to produce a response in cells in a fashion similar to the wild-type protein. For instance, such variant forms of a TLR11 polypeptide can be assessed, e.g., for their ability to activate NF-κB; e.g., to stimulate the production of cytokines such as for example, TNF-α; e.g., to bind to another polypeptide such as for example, another TLR11 polypeptide or another protein involved in immunomodulatory activity. Polypeptides in which more than one replacement has taken place can readily be tested in the same manner.

This invention further contemplates a method of generating sets of combinatorial mutants of the subject TLR11 polypeptides, as well as truncation

mutants, and is especially useful for identifying potential variant sequences (e.g., homologs) that are functional in binding to a TLR11 polypeptide. The purpose of screening such combinatorial libraries is to generate, for example, TLR11 homologs which can act as either agonists or antagonist, or alternatively, which possess novel activities all together. Combinatorially-derived homologs can be generated which have a selective potency relative to a naturally occurring TLR11 polypeptide. Such proteins, when expressed from recombinant DNA constructs, can be used in gene therapy protocols.

Yet another aspect of the present invention concerns an immunogen which comprises a TLR11 polypeptide capable of eliciting an immune response specific for the TLR11 polypeptide; e.g., a humoral response, an antibody response; or a cellular response. In certain embodiments, the immunogen comprises an antigenic determinant, e.g., a unique determinant, from a protein represented by SEQ ID NO:2.

Another aspect of the invention pertains to an antibody specifically reactive with a TLR11 polypeptide. For example, by using immunogens derived from a TLR11 polypeptide, e.g., based on the cDNA sequences, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols. A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide (e.g., a TLR11 polypeptide or an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein as described above). Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of a TLR11 polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies. In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of a TLR11 polypeptide of a mammal, e.g., antigenic determinants of a protein set forth in SEQ ID NO:2.

In another embodiment, the antibodies are immunoreactive with one or more proteins having an amino acid sequence that is at least 70% identical, at least 80%

identical to an amino acid sequence as set forth in SEQ ID NO:2. In other embodiments, an antibody is immunoreactive with one or more proteins having an amino acid sequence that is 75%, 80%, 85%, 90%, 95%, 98%, 99% or identical to an amino acid sequence as set forth in SEQ ID NO:2.

Following immunization of an animal with an antigenic preparation of a TLR11 polypeptide, anti-TLR11 antisera can be obtained and, if desired, polyclonal anti-TLR11 antibodies isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) Nature, 256: 495-497), the human B cell hybridoma technique (Kozbar et al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a mammalian TLR11 polypeptide of the present invention and monoclonal antibodies isolated from a culture comprising such hybridoma cells. In one embodiment, anti-mouse TLR11 antibodies specifically react with the protein encoded by a nucleic acid having SEQ ID NO: 1.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with one of the subject TLR11 polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab)_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab)_2$ fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific, single-chain, and chimeric and humanized molecules having affinity for a TLR11 polypeptide conferred by at least one CDR region of the antibody. In certain embodiments, the antibody further comprises a label attached thereto and able to be detected, (e.g., the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor).

An application of anti-TLR11 antibodies of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as gt11, gt18-23, ZAP, and ORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins. For instance, gt11 will produce fusion proteins whose amino termini consist of ß-galactosidase amino acid sequences and whose carboxy termini consist of a foreign polypeptide. Antigenic epitopes of a TLR11 polypeptide, e.g., other orthologs of a particular protein or other paralogs from the same species, can then be detected with antibodies, as, for example, reacting nitrocellulose filters lifted from infected plates with the appropriate anti-TLR11 antibodies. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of TLR11 homologs can be detected and cloned from other animals, as can alternate isoforms (including splice variants) from humans.

In certain embodiments, the present invention provides assays for identifying therapeutic agents which either interfere with or promote TLR11 function. In certain embodiments, agents of the invention modulate the activity of TLR11 and may be used to treat certain diseases related to an inflammatory disorder, an autoimmune disease, a cardiovascular disorder, or a systemic infection that is responsive to Toll-like receptor modulation. In certain embodiments, agents of the invention modulate the activity of TLR11 and may be used to treat a viral, fungal or bacterial infection, including urinary tract infections; asthma; rhinitis; chronic obstructive pulmonary disease (COPD); emphysema; an inflammatory bowel disease such as ulcerative colitis or Crohn's disease; rheumatoid arthritis; osteoarthritis; psoriasis; Alzheimers disease; atherosclerosis, Multiple Sclerosis; diabetes; and septic shock syndrome associated with systemic infection involving gram positive or gram negative bacteria. In certain embodiments, the invention provides assays to identify, optimize or otherwise assess agents that increase or decrease the activity of a TLR11 polypeptide.

In certain embodiments, an assay comprises screening for activation of NF-κB. For example, mammalian cells such as HEK293 cells transfected with an NF-κB luciferase reporter construct and expressing a constitutively active TLR11 polypeptide or TLR11 fusion protein (e.g., the cytoplasmic domain of TLR11 fused

to the extracellular domain of a CD4 receptor) are assayed for NF-κB activation. For instance, activation of NF-κB by constitutively active TLR11 is measured by NF-κB induced luciferase activity which is measured by means of a luminometer. The above assay can similarly be conducted by assaying for the activation of AP1.

Alternatively, in certain embodiments, an assay comprises screening for activation of NF-κB by TLR11 polypeptides activated by means of an agent such as an endogenous ligand or a therapeutic compound. For example, mammalian cells such as HEK293 cells are transfected with an NF-κB luciferase reporter construct and express a TLR11 polypeptide. The TLR11 polypeptide is contacted with an agent such as the supernatant from a uropathogenic bacterial culture which activates TLR11. TLR11 activation by the agent is measured by the activation of NF-κB, which activity is measured by luciferase activity by means of a luminometer.

Alternatively, an assay comprises detecting the production of cytokines. For example, mammalian cells such as RAW 264.7 macrophages expressing a constitutively active TLR11 or TLR11 fusion protein (e.g., the cytoplasmic domain of TLR11 fused to the extracellular domain of a CD4 receptor) are tested for production of the cytokine, TNF-α, at the cell surface of the cells by immunostaining for TNF-α followed by flow cytometry.

An assay as described above may be used in a screening assay to identify agents that modulate an immunomodulatory activity of a TLR11 polypeptide. A screening assay will generally involve adding a test agent to one of the above assays, or any other assay designed to assess an immunomodulatory-related activity of a TLR11 polypeptide. The parameters detected in a screening assay may be compared to a suitable reference. A suitable reference may be an assay run previously, in parallel or later that omits the test agent. A suitable reference may also be an average of previous measurements in the absence of the test agent. In general the components of a screening assay mixture may be added in any order consistent with the overall activity to be assessed, but certain variations may be preferred.

In a screening assay, the effect of a test agent may be assessed by, for example, assessing the effect of the test agent on kinetics, steady-state and/or endpoint of the reaction.

Certain embodiments of the invention relate to assays for identifying agents that bind to a TLR11 polypeptide, optionally a particular domain of TLR11 such as an extracellular domain (e.g., a leucine rich repeat domain) or an intracellular domain such as a TIR domain. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, and immunoassays for protein binding. The purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions and design of test agents. In one embodiment, an assay detects agents which inhibit the activation of one or more subject TLR11 polypeptides. In another embodiment, the assay detects agents which modulate the intrinsic biological activity of a TLR11 polypeptide, such as activation of NF-κB or stimulation of the production of cytokines (e.g., TNF-α).

Assay formats which approximate such conditions as formation of protein complexes, enzymatic activity, and TLR11 immunomodulatory activity, e.g., purified proteins or cell lysates, as well as cell-based assays which utilize intact cells. Simple binding assays can also be used to detect agents which bind to TLR11. Such binding assays may also identify agents that act by disrupting the interaction between a TLR11 polypeptide and a TLR11 interacting protein, or the binding of a TLR11 polypeptide or complex to a substrate. Agents to be tested can be produced, for example, by bacteria, yeast or other organisms (e.g., natural products), produced chemically (e.g., small molecules, including peptidomimetics), or produced recombinantly. In one embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

In a further embodiment, the invention provides an assay for identifying a test compound which inhibits or potentiates the activation of a TLR11 polypeptide, comprising: (a) forming a reaction mixture including TLR11 polypeptide and a test compound; and (b) detecting activation of said TLR11 polypeptide; wherein a change in the activation of said TLR11 polypeptide in the presence of the test compound, relative to activation in the absence of the test compound, indicates that said test compound potentiates or inhibits activation of said TLR11 polypeptide.

Assaying TLR11 complexes, in the presence and absence of a candidate inhibitor, can be accomplished in any vessel suitable for containing the reactants. Examples include microtitre plates, test tubes, and micro-centrifuge tubes.

In one embodiment of the present invention, drug screening assays can be generated which detect inhibitory agents on the basis of their ability to interfere with assembly or stability of the TLR11 complex. In an exemplary binding assay, the compound of interest is contacted with a mixture comprising a TLR11 polypeptide and at least one interacting polypeptide. Detection and quantification of TLR11 complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) interaction between the two polypeptides. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. In the control assay, the formation of complexes is quantitated in the absence of the test compound.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays of the present invention which are performed in cell-free systems, such as may be developed with purified or semi-purified proteins or with lysates, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with other proteins or changes in enzymatic properties of the molecular target.

The present invention is illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE 1. Features of the TLR 11 sequence

A mouse cDNA library from RAW macrophages was screened with primers designed based on expressed sequence tags ("ESTs") identified in the EST database

available from the National Center for Biotechnology Information ("NCBI"). A novel Toll-like receptor, TLR11, was cloned. The cDNA sequence of TLR11 is depicted in Figure 1A, and the amino acid sequence of TLR11 is depicted in Figure 1B. The predicted signal peptide comprises residues 1 to 30 and the trans-membrane segment comprises residues 705 to 729. An alignment of the amino acid sequence of cytoplasmic domains of known Toll like receptor family members with TLR1-11 is shown in Figure 1C. Alignments were performed using the Clustal algorithm and boxshade. Three regions (box 1, 2 and 3 in Figure 1B) are conserved across all TIR domains and are thought to be important for signaling.

Multiple tissue Northern analysis was conducted in order to determine the expression pattern of TLR 11 mRNA (Figure 1D). TLR11 is predominantly expressed in kidney and liver with significantly lower level of expression in spleen and heart. A β-actin probe was used as a control for RNA loading.

Localization of TLR11 mRNA in tissues was determined by in situ hybridization (Figure 1E). TLR11 localization is shown by incubation with the antisense riboprobe of TLR11 in medulla (a) and cortex (c) of kidney, and liver (e). Control incubations using TLR11 sense riboprobe were negative (b, d, f).

EXAMPLE 2 TLR11 induced activation of transcription was measured by reporter gene expression and endogenous cytokine.

Constitutively active TLR11 was shown to activate NF-κB and AP1 (Figures 2A and B). 293 cells were transiently transfected with expression vectors for CD4/TLR11 or CD4/TLR4 fusion constructs. In these constructs the cytosolic domain of the TLRs (the TIR domain) was fused to the extracellular portion of the CD4 receptor. When the extracellular portion of CD4 is overexpressed, it aggregates, and can be used to stimulate downstream signaling pathways independent of ligand activation. The amount of DNA transfected was equalized with empty expression vector, which was also used in the control together with either an NF-κB or AP1 luciferase reporter construct. NF-κB and AP1 induced luciferase activity were measured using a luminometer.

Transfection of RAW 264.7 macrophages was carried out with a CD4/TLR11 expression vector. The production of the cytokine, TNF-α, a key

marker of innate immune responses, was detected by immunostaining for cell surface TNF followed by flow cytometry. The dark gray region indicates TNF-α expression in untransfected cells, whereas the light gray line represents TNF-α produced in cells transfected with CD4/TLR11. (See Figure 2C).

Dominant-negative MyD88 (DN-MyD88), dominant-negative IRAK (DN-IRAK), and dominant-negative TRAF6 (DN-TRAF6) constructs were shown to inhibit CD4/TLR11 mediated NF-κB activation (Figures 2D and E). 293-luc cells, stably transfected cells with the NF-κB luciferase reporter construct, were co-transfected with CD4/TLR11 and wild-type or dominant-negative versions of MyD88, IRAK and TRAF6. MyD88, IRAK and TRAF6 are inhibitors of Toll-signaling.

Tollip, a physiological inhibitor of Toll-signaling, was shown to inhibit CD4/TLR11 induced NF-κB activation (Figure 2F). Tollip was cotransfected into 293-luc cells along with CD4/TLR11.

EXAMPLE 3. Generation and characterization of cell lines stably expressing TLR11.

Human 293 cells were cultured in DMEM, 7% fetal calf serum (Gemini), Pen/Strep (Life Technologies), glutamine (Life Technologies). The stable cell line 293κB LUC was made by cotransfecting the NF-κB reporter gene, pBIIxLUC (Kopp and Ghosh 1994) and the plasmid, pCI-neo (Promega) (at a ratio of 10:1 respectively) into 293 cells using Lipofectamine (GIBCO/BRL, manufacturers instructions). Stable transfectants were selected with G418 (Life Technologies) at 1.6mg/ml. Positive clones were assayed by treatment of cells for 5 hours with IL-1β (human recombinant, GENZYME) followed by luciferase assay (Promega). The cell line used can be stimulated approximately 50 fold in such assays.

TLR11 expression constructs were made by inserting PCR-generated TLR 11 cDNA, lacking the signal peptide sequence, into pFLAG-CMV-1 vector (Sigma). To generate TLR11-expressing stable cell lines, 293-Luc cells were seeded into 10-cm dishes and transfected using FuGene6 (Roche) with 5 μg of TLR11/pFLAG construct together with 0.5 μg of a plasmid expressing a hygromycin resistance gene. Cells were selected in Dulbecco's modified Eagle's medium with 200μg/ml

hygromycin B (Calbiochem). Individual colonies were picked, expanded, and expression of TLR11 was confirmed by immunoblotting. Cell-surface expression of TLR11 was examined by flow cytometry (FACS) using anti-FLAG M2 antibody. To permeabilize the cells the fluorochrome-labeled antibody was dissolved in 0.3% saponin in PBS to the working dilution. The cells were incubated with the fluorochrome-antibody/saponin for 30 minutes at 40C, and then the cells were washed with PBS before subjecting them to FACS. Stable cell lines were propagated and maintained in high glucose Dulbecco's modified Eagle's medium supplemented with 7% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Six different, independently derived cell lines stably expressing TLR11/pFlag in 293 cells transfected with the κB-luciferase reporter (293-luc cells) were obtained. Expression of TLR11 was confirmed by immunoblotting (Figure 3A).

Cell surface expression of TLR11 in the stable cell line was detected using FACS (Figure 3B). The dark gray region indicates untransfected cells, whereas light gray indicates cells transfected with TLR11/pFlag.

EXAMPLE 4 Uropathogenic bacteria contain TLR11-stimulating activity.

Human embryonic kidney 293 cells (5 x 104) were transiently transfected using FuGene6 plus 0.2 μg NF-κB-dependent luciferase reporter pBIIX construct together with 1μg of constructs expressing the different Flag-tagged TLRs (TLR2, TLR4, TLR5, TLR11 or empty expression vectors), and plated into 24-well tissue culture plates. Twenty-four hours after transfection, cells were stimulated as indicated, with 100 ng/ml peptidoglycan or 100 ng/ml LPS, 100 ng/ml Flagellin, 100 ng/ml dsRNA, or 100 ng/ml CpG DNA or 70 μl/ml heat-killed supernatant from the indicated saturated bacterial cultures for 6h, and luciferase activity was measured in a luminometer according to the manufacturer's instructions (Promega). Data are representative of three independent experiments. (See Figures 4A and B). The uropathogenic bacterial strains are E.coli(U) and Klebsiella. DH5α is not known to be uropathogenic.

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid sequence selected from the group consisting of: a nucleic acid which is represented by SEQ ID NO: 1; a nucleic acid sequence that is at least 70% identical to the nucleic acid sequence of SEQ ID NO: 1; a nucleic acid sequence which is represented by the complement to SEQ ID NO: 1; and a nucleic acid sequence that is at least 70% identical to the complement of the nucleic acid sequence represented by SEQ ID NO: 1.

2. An isolated nucleic acid that hybridizes under high stringency conditions to the nucleic acid represented by SEQ ID NO: 1 or to its complement.

3. An isolated nucleic acid comprising a nucleic acid sequence that, due to the degeneracy of the genetic code, encodes the amino acid sequence encoded by the nucleic acid sequence depicted in SEQ ID NO: 1.

4. An isolated Toll-like receptor polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence that is at least 70% identical to the amino acid sequence depicted in SEQ ID NO: 2; an isolated Toll-like receptor polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence depicted in SEQ ID NO: 2; and an isolated Toll-like receptor polypeptide comprising an amino acid sequence that is represented by SEQ ID NO: 2.

5. The isolated polypeptide of claim 4, wherein the isolated polypeptide is a variant of a polypeptide represented by SEQ ID NO: 2.

6. The isolated polypeptide of claim 4, wherein the isolated polypeptide is a fragment of a polypeptide represented by SEQ ID NO: 2.

7. A vector comprising nucleic acid sequence encoding a polypeptide that is at least 70% identical to the polypeptide represented by SEQ ID NO: 2.

8. The vector of claim 7, wherein the nucleic acid is operably linked to a transcriptional regulatory sequence.

9. Isolated host cells comprising exogenous nucleic acid encoding a polypeptide that is at least 70% identical to the polypeptide represented by SEQ ID NO: 2.

10. The isolated host cells of claim 9, wherein the exogenous nucleic acid is a vector.

11. The isolated host cells of claim 10, wherein the vector is a vector of claim 8.

12. A method of producing a TLR11 polypeptide comprising culturing the host cells of claim 11 under conditions suitable for expression of the TLR11 polypeptide, wherein the TLR11 polypeptide is thereby produced.

13. A monoclonal or polyclonal antibody, or a chimera or fragment thereof, which is specifically reactive with an epitope of a polypeptide of claim 4.

14. A method for identifying compounds which modulate TLR11 activity comprising:

(a) contacting a polypeptide according to claim 4 with a test agent; and

(b) monitoring for modulation of TLR11 activity,

wherein a compound which modulates TLR11 activity is thereby identified.

15. The method of claim 14, wherein the TLR11 activity monitored in step (b) is NF-κB activation.

16. The method of claim 14, wherein the TLR11 activity monitored in step (b) is AP1 activation.

17. The method of claim 14, wherein the TLR11 activity monitored in step (b) is the production of cytokines.

18. The method of claim 17, wherein the cytokine is TNF-α.

19. A compound identified a method according to claim 14.

20. A method of treating an individual having a disorder that is responsive to Toll-like receptor modulation, which method comprises administering to the individual an effective amount of a compound according to claim 19 or an antibody according to claim 13.

21. The method of claim 20, wherein the disorder is selected from the group consisting of: an inflammatory disorder, an autoimmune disease, a cardiovascular disorder, and a systemic infection.

22. The method according to claim 21, wherein the disorder is selected from the group consisting of: a viral, fungal or bacterial infection, including urinary tract infections; asthma; rhinitis; chronic obstructive pulmonary disease (COPD); emphysema; an inflammatory bowel disease such as ulcerative colitis or Crohn's disease; rheumatoid arthritis; osteoarthritis; psoriasis; Alzheimers disease; atherosclerosis, Multiple Sclerosis, diabetes; and septic shock syndrome associated with systemic infection involving gram positive or gram negative bacteria.

23. A polypeptide according to claim 4 for use as an adjuvant.

24. The use of a compound according to claim 19 in the manufacture of a medicament for the treatment of a disorder that is responsive to Toll-like receptor modulation.

Figure 1A

TLR 11 cDNA sequence

ATGGGCAGGTACTGGCTGCTGCCAGGTCTCCTCCTTTCCCTGCCTCTGGTAACTGGGT
GGAGCACTTCCAACTGCCTGGTGACCGAAGGCTCCCGACTGCCCCTGGTCTCCCGCT
ATTTCACATTCTGCCGCCATTCCAAGCTATCCTTTCTTGCTGCATGCCTCTCCGTGAGC
AACCTGACACAGACCTTGGAAGTTGTACCTCGGACTGTGGAGGGGCTCTGCCTCGGT
GGTACTGTGTCTACTCTGCTTCCAGATGCTTTCTCTGCTTTTCCTGGTCTCAAGGTCCT
GGCACTGAGTCTGCACCTTACCCAACTTCTGCCAGGAGCTCTCCGGGGTCTGGGACA
GTTGCAGAGCCTCTCTTTTTTTGACTCTCCTCTTAGGAGATCTCTCTTTCTACCTCCTG
ATGCCTTCAGTGACCTGATTTCCCTCCAGAGACTCCATATCTCTGGCCCTTGCCTGGA
TAAGAAGGCAGGCATCCGCCTGCCTCCCGGTCTGCAATGGCTGGGTGTCACGCTCAG
TTGCATTCAGGACGTGGGAGAGCTGGCTGGTATGTTCCCAGATCTGGTGCAAGGTTC
CTCCTCCAGGGTTTCGTGGACCCTGCAGAAGTTGGATCTGTCATCCAACTGGAAGCT
GAAGATGGCTAGTCCTGGGTCCCTCCAGGGTCTCCAGGTGGAGATTCTGGACCTGAC
AAGAACACCACTGGATGCCGTGTGGCTGAAGGGCCTGGGACTTCAGAAACTCGATGT
CTTGTATGCACAGACTGCCACGGCCGAGCTGGCTGCTGAGGCTGTTGCCCACTTTGA
GCTGCAGGGCTTGATTGTGAAAGAAAGCAAGATAGGATCTATATCTCAGGAGGCTCT
GGCTTCCTGCCACAGCCTGAAGACCTTGGGTCTTTCAAGCACTGGCCTAACCAAGCT
TCCACCAGGCTTCCTGACTGCCATGCCTAGGCTTCAGCGACTGGAGCTGTCCGGAAA
CCAACTGCAGAGCGCCGTGCTGTGCATGAATGAGACGGGAGATGTGTCAGGACTCA
CGACTCTGGATCTGTCAGGCAACAGGTTGCGCATCCTGCCTCCAGCCGCCTTCTCCTG
CTTACCCCACTTGCGAGAGCTGCTGCTTCGGTACAACCAGCTGCTTTCCCTGGAGGG
ATACCTATTCCAGGAGCTCCAGCAACTAGAGACCTTGAAGCTGGATGGAAACCCCCT
GCTTCACCTGGGTAAGAACTGGTTGGCGGCTCTGCCTGCATTGACCACCCTTAGCTTG
CTAGATACCCAAATACGGATGAGCCCAGAGCCTGGCTTCTGGGGAGCAAAGAATCT
GCATACCTTGAGCCTGAAGCTTCCCGCTCTCCCTGCTCCGGCAGTATTGTTCCTGCCC
ATGTATCTGACCAGCTTAGAGCTTCATATAGCCTCAGGCACGACGGAGCACTGGACG
CTGTCCCCAGCGATCTTTCCTTCCTTGGAGACCTTGACTATAAGCGGCGGGGGACTG
AAGCTGAAGCTGGGGTCCCAGAATGCTTCTGGGGTCTTCCCTGCTCTCCAGAAGCTC
TCCCTGCTTAAGAACAGCTTGGATGCCTTCTGCTCCCAGGGTACCTCCAACCTTTTCC
TCTGGCAGCTCCCCAAACTTCAGTCCTTGAGGGTATGGGGTGCTGGAAACAGCTCCA
GACCCTGCCTTATCACTGGGCTGCCCAGCCTACGGGAGCTGAAGCTGGCGTCGCTTC
AGTCCATAACCCAGCCCCGTTCGGTGCAGCTGGAGGAGCTGGTGGGTGACCTTCCAC
AGCTCCAGGCCTTAGTGCTATCCAGCACAGGCCTCAAGTCACTGTCGGCTGCTGCTTT
CCAGCGCCTGCACAGTCTCCAGGTCTTAGTGCTAGAATACGAGAAGGACTTGATGCT
GCAGGACAGTCTGAGGGAGTACAGCCCTCAGATGCCCCACTATATATACATTCTGGA
GTCAAACCTGGCCTGCCACTGTGCCAATGCGTGGATGGAGCCATGGGTTAAGCGGTC
CACTAAAACGTACATATACATAAGAGACAATCGCTTATGTCCAGGACAAGACAGGCT
CTCTGCTAGGGGTTCCCTTCCCTCCTTTCTCTGGGACCACTGCCCCCAGACGTTGGAG
CTGAAACTCTTTTTGGCTAGTTCTGCCTTGGTGTTCATGCTAATTGCCTTGCCTCTCCT
CCAAGAAGCCAGGAACTCTTGGATCCCCTACCTGCAGGCCTTGTTCAGGGTTTGGCT
CCAGGGTCTGAGGGGTAAGGGAGACAAGGGGAAGAGGTTCCTTTTTGATGTATTCGT
GTCCCACTGCAGGCAAGACCAGGGCTGGGTGATAGAGGAACTTCTGCCTGCTCTGGA
GGGCTTCCTTCCAGCTGGCCTGGGCCTGCGCCTCTGTCTCCCCGAGCGTGACTTTGAG
CCTGGTAAGGATGTAGTTGATAATGTGGTAGATAGCATGTTGAGCAGCCGTACCACA
CTCTGCGTGTTGAGTGGGCAGGCCCTGTGTAACCCCCGATGCCGCCTGGAGCTCCGC
TTGGCCACCTCTCTCCTCCTGGCTGCCCCGTCCCCTCCAGTGTTGCTGCTAGTCTTCTT
GGAACCCATTTCTCGGCACCAGCTTCCGGGTTACCACAGACTGGCTCGGCTGCTTCG
AAGAGGAGACTACTGTCTGTGGCCCGAGGAAGAGGAGAGAAAGAGTGGGTTCTGGA
CTTGGCTGAGGAGCAGGCTAGGGTAG

Figure 1B

MGRYWLLPGLLLSLPLVTGWSTSNCLVTEGSRLPLVSRYFTFCRHSKLSFLAACLSVSNLTQTLEVVPRTVEGLCL
GGTVSTLLPDAFSAFPGLKVLALSLHLTQLLPGALRGLGQLQSLSFFDSPLRRSLFLPPDAFSDLISLQRLHISGP
CLDKKAGIRLPPGLQWLGVTLSCIQDVGELAGMFPDLVQGSSSRVSWTLQKLDLSSNWKLKMASPGSLQGLQVEIL
DLTRTPLDAVWLKGLGLQKLDVLYAQTATAELAAEAVAHFELQGLIVKESKIGSISQEALASCHSLKTLGLSSTGL
TKLPPGFLTAMPRLQRLELSGNQLQSAVLCMNETGDVSGLTTLDLSGNRLRILPPAAFSCLPHLRELLLRYNQLLS
LEGYLFQELQQLETLKLDGNPLLHLGKNWLAALPALTTLSLLDTQIRMSPEPGFWGAKNLHTLSLKLPALPAPAVL
FLPMYLTSLELHIASGTTEHWTLSPAIFPSLETLTISGGGLKLKLGSQNASGVFPALQKLSLLKNSLDAFCSQGTS
NLFLWQLPKLQSLRVWGAGNSSRPCLITGLPSLRELKLASLQSITQPRSVQLEELVGDLPQLQALVLSSTGLKSLS
AAAFQRLHSLQVLVLEYEKDLMLQDSLREYSPQMPHYIYILESNLACHCANAWMEPWVKRSTKTYIYIRDNRLCPG
QDRLSARGSLPSFLWDHCPQTLELKLFLASSALVFMLIALPLLQEARNSWIPYLQALFRVWLQGLRGKGDKGKRFL
FDVFVSHCRQDQGWVIEELLPALEGFLPAGLGLRLCLPERDFEPGKDVVDNVVDSMLSSRTTLCVLSGQALCNPRC
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Figure 1C

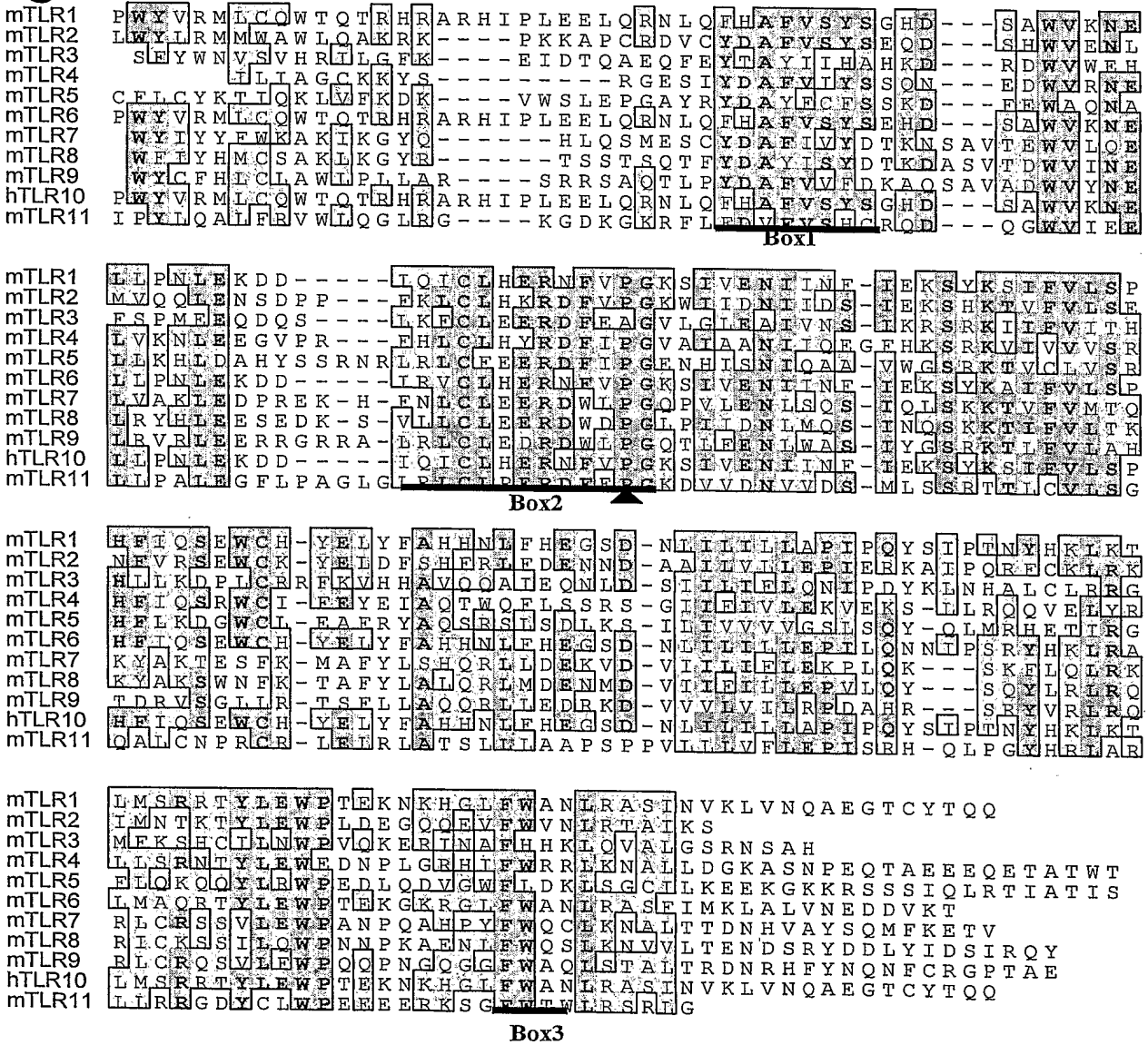


Figure 1D

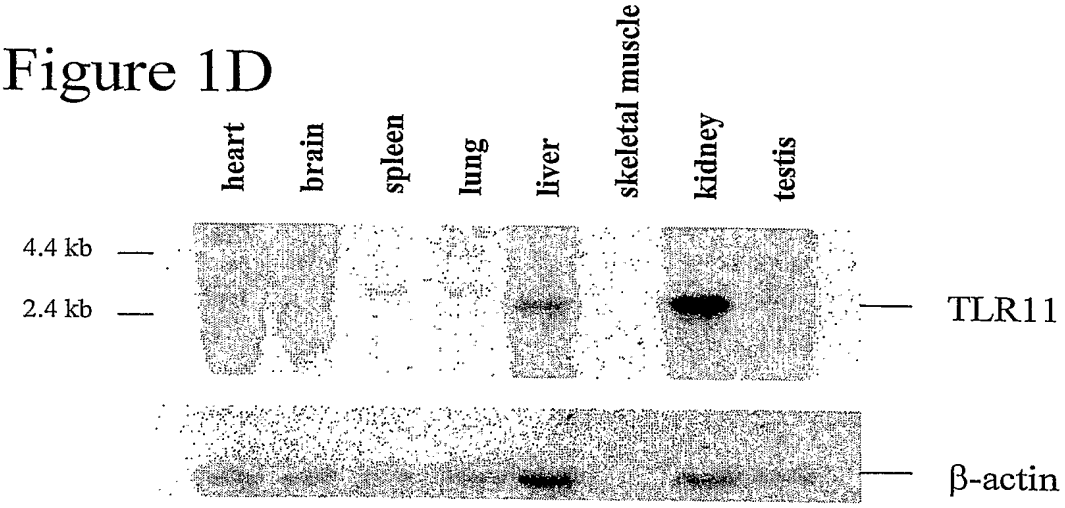


Figure 1E

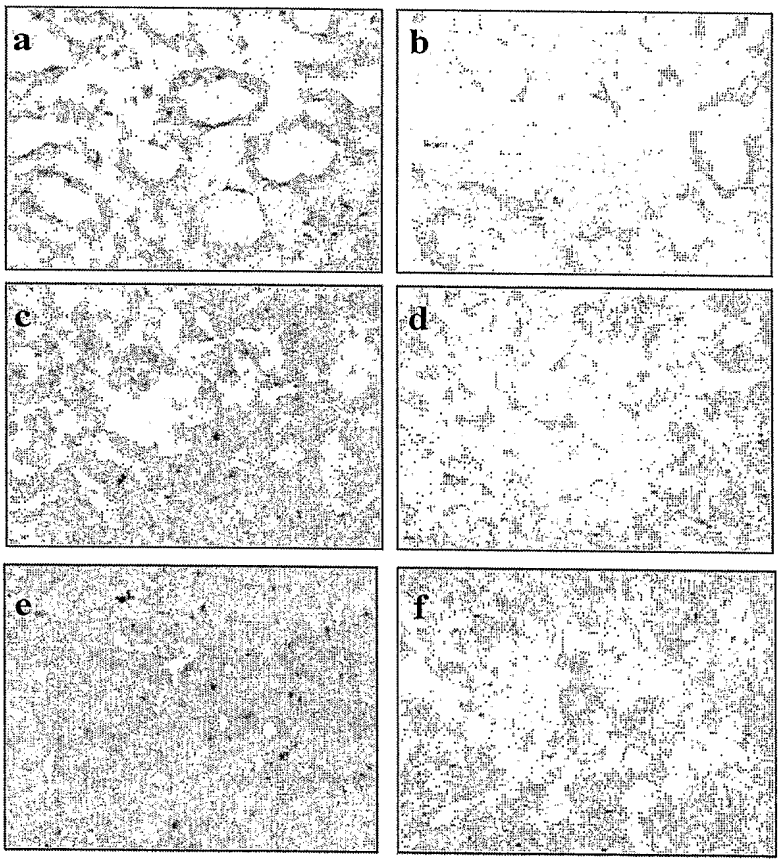


Figure 2A

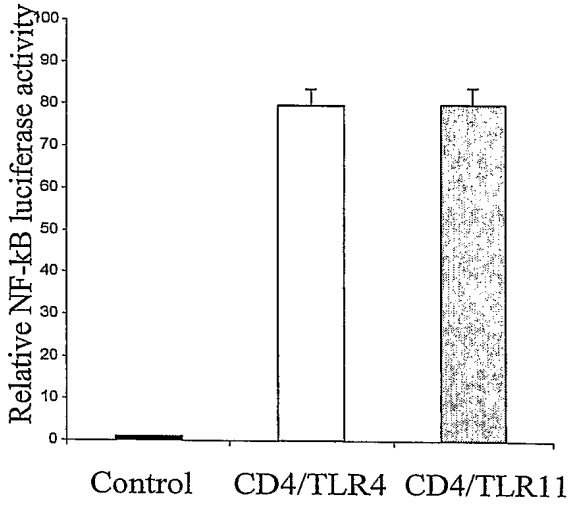


Figure 2B

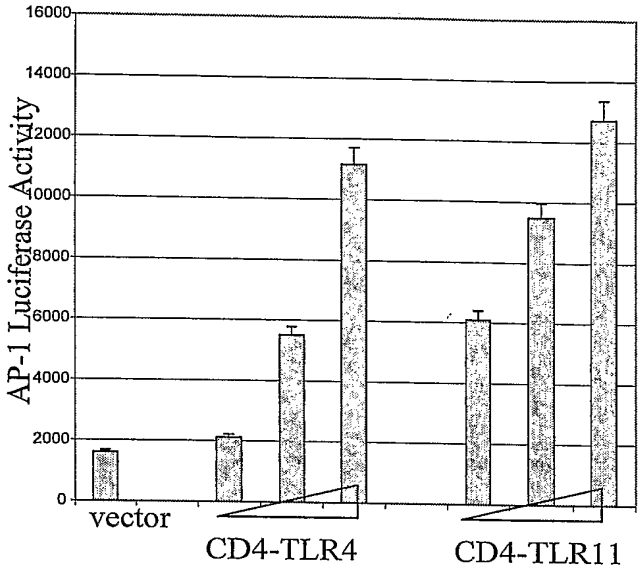


Figure 2C

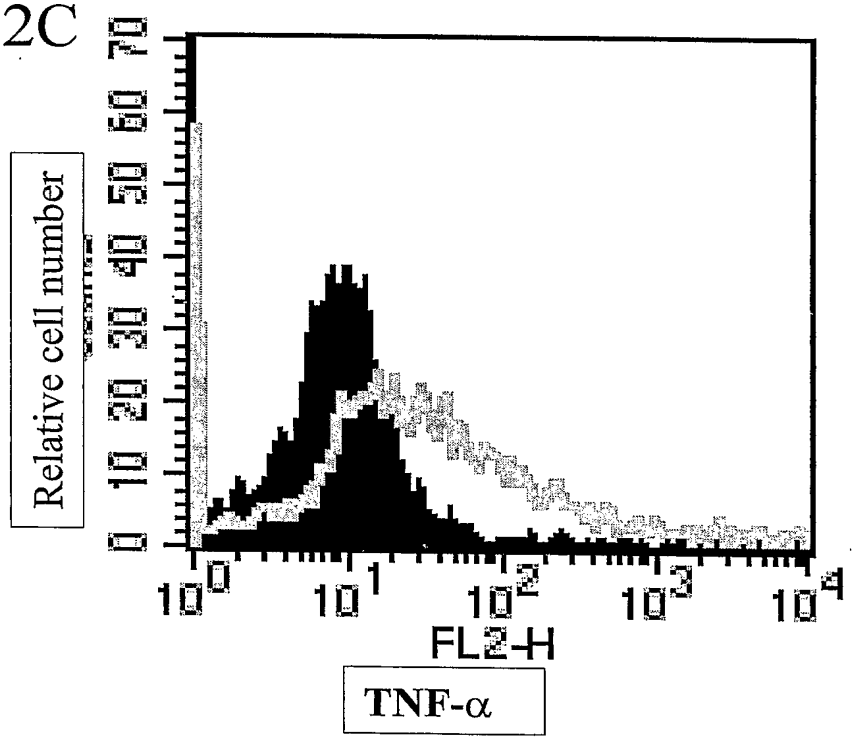


Figure 2D

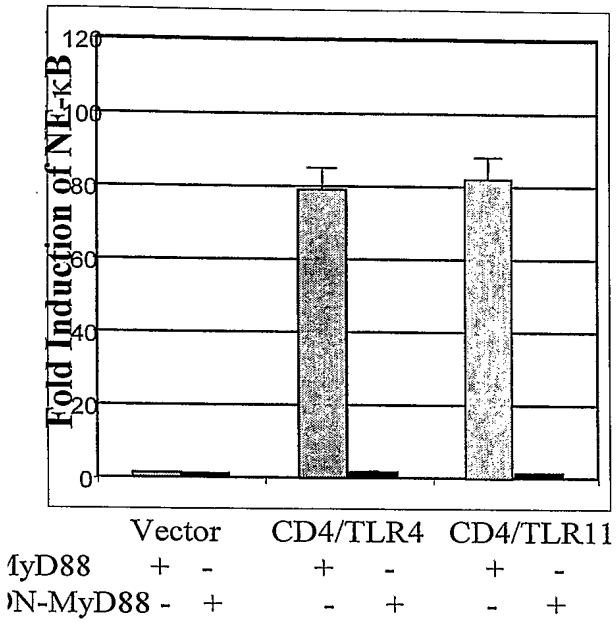


Figure 2E

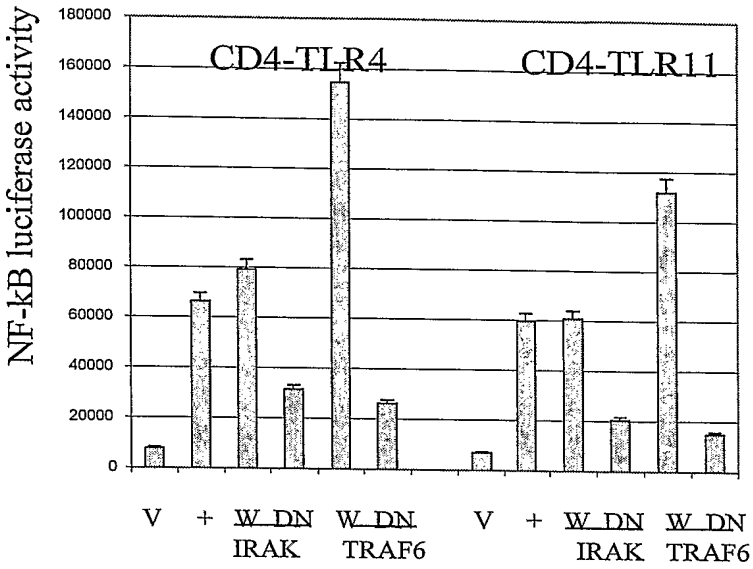


Figure 2F

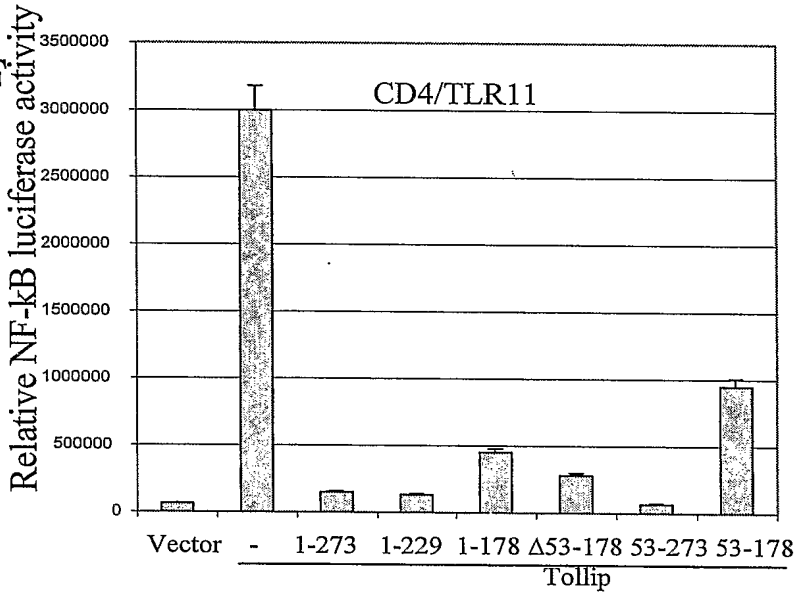


Figure 3A

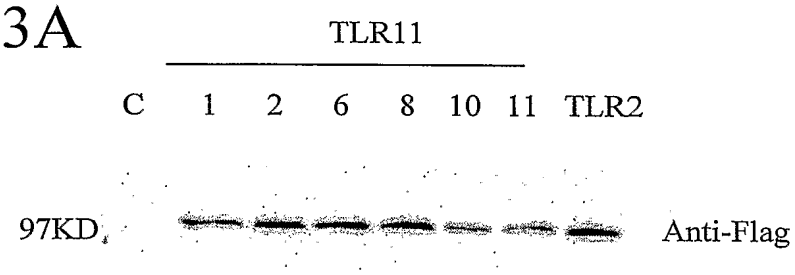


Figure 3B

293 cells

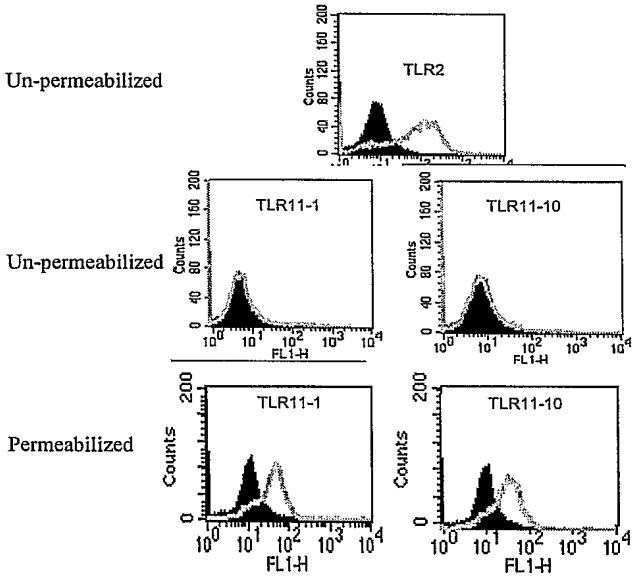


Figure 4A

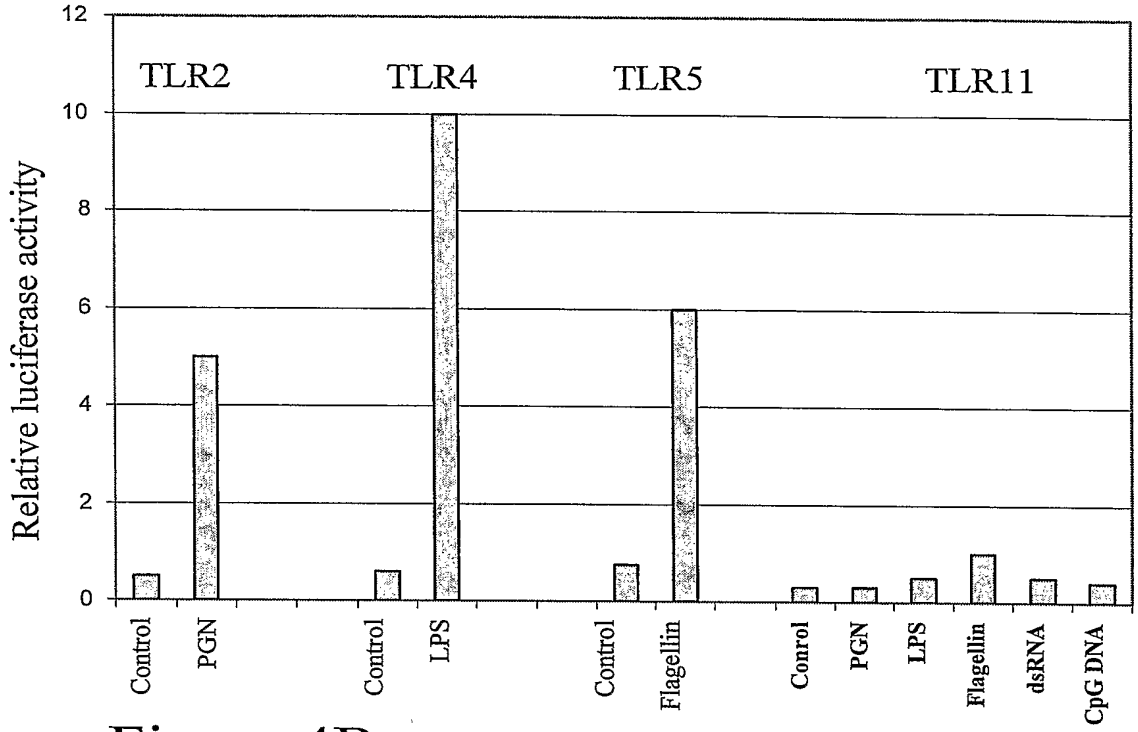


Figure 4B

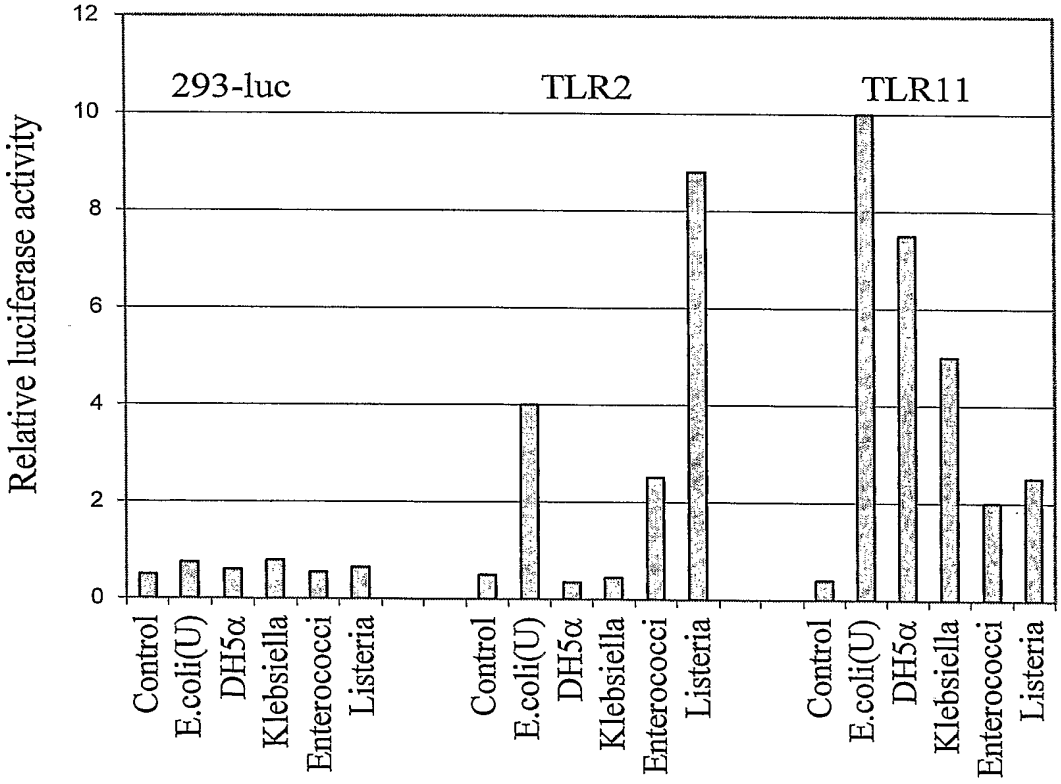
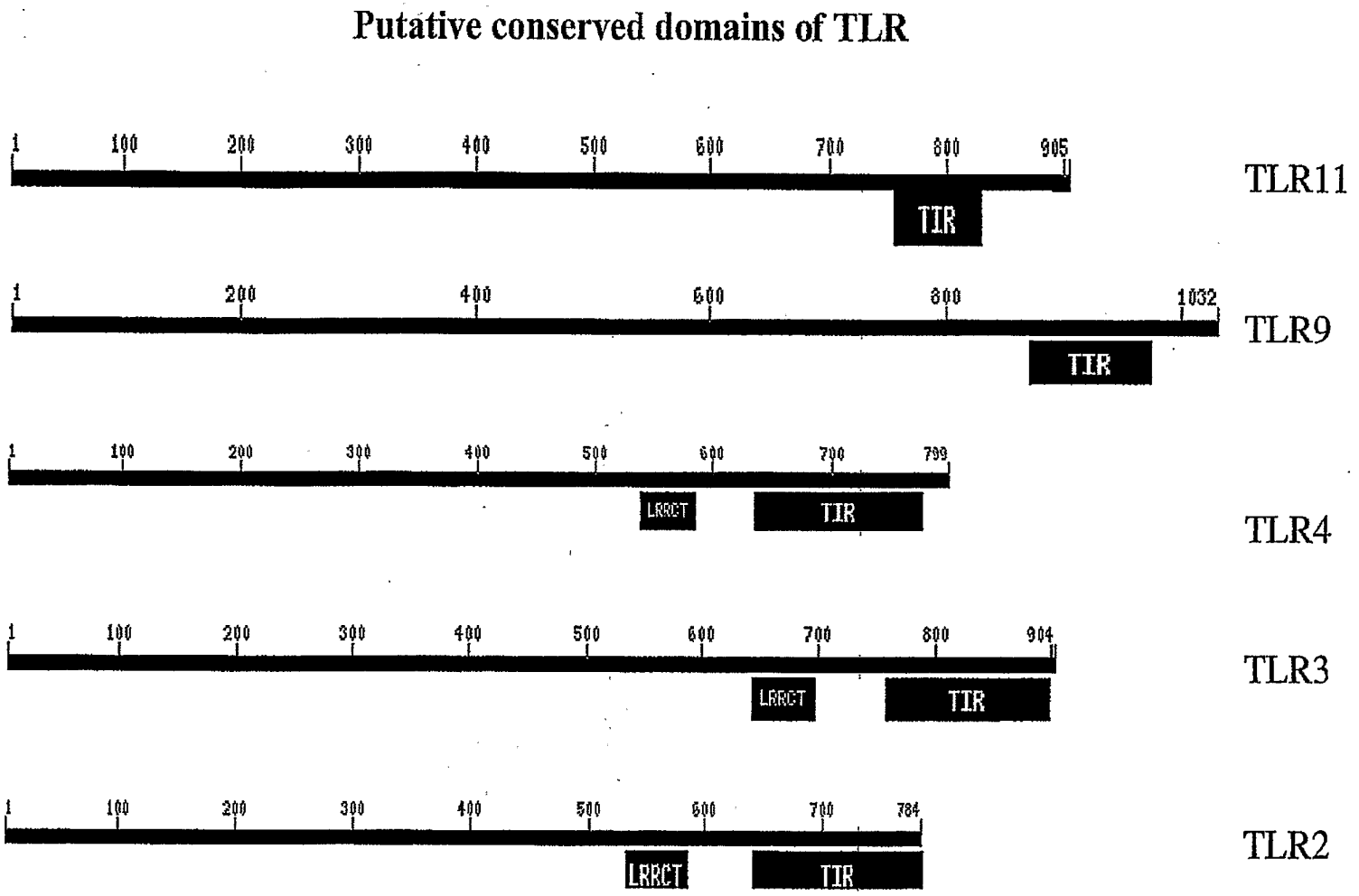


Figure 5


Putative conserved domains of TLR
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